Pharmaceutical Research, Vol. 3, No. 1, 1986

Commentary

Protein Drugs: A Revolution in Therapy?

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Miracle cures are just on the horizon—or so it seems if one follows the incessant news releases touting yet another breakthrough in the biomedical sciences. Much of the public attention focuses on novel proteins with powerful biological properties that mediate and regulate interactions among various tissues of the body. Sometimes referred to under the pretentious name "biological response modifiers"—I would prefer a simpler term, such as the *cell signal proteins*—these substances have several features in common: They act in exceedingly low concentrations to affect crucial functions of the body—hemostasis, the immune response, the endocrine system, neural development, among others. Further, they are transmitted from one cell to another with the active species, hence, circulating in the extracellular fluid that can also be reached by the exogenously administered substance. Once cloning of the corresponding gene has been accomplished through recombinant DNA technology, these signal proteins are instantly available in large quantities. As most of these factors are highly conserved and derived from cloned human genes, potential immunogenicity is minimized.

Expectations are high, the therapeutic potential staggering. But is it not premature to invoke a revolution in therapy? Revolution suggests profound and violent changes of an entire system, in this case the health care system, which consumes a shocking 10% of the entire gross national product in the USA. The formula, biotechnology plus cell biology yield novel therapies, indeed rocks the very fabric of the health care system, affecting medical, scientific, economic, legal, and public issues. Market analysts estimate the total investment into biotechnology ventures approaches $2.5 billion, spawning some 300 biotech companies in the USA alone. Among the three major thrusts of this biotechnology boom: (1) human diagnostics and therapeutic drugs, (2) process engineering and instrumentation, (3) agriculture/animal health, pharmaceutical developments take a prominent place. While the exceedingly long lag time and high expense of pushing a new therapeutic agent through the entire drug approval process have dried up some of the venture capital and an industry shakeout appears likely, at least the two frontrunners, Genentech and Cetus from California, have each set their course on growing into a full-fledged pharmaceutical company. If successful, they would be the first to break into the major pharmaceutical ranks since the success of Syntex Corporation, propelled by marketing the birth control pill more than 25 years ago. Meanwhile, nearly all major drug companies and industrial giants, such as Shell, Monsanto, and Dupont, are buying into small biotechnology firms to secure part of the expected benefits.

Next to economic upheaval, new legal problems emerge. Can living organisms or cells be patented? What would be the value of such patents if they can be readily circumvented with minor modifications of therapeutic proteins? A rather small but somewhat bizarre legal case comes to mind: A patient sues the University of California after medical researchers have transformed cells from his blood into an established line that produces valuable substances, such as interferon. The patient claims he should be a beneficiary of the University's patent rights and licensing agreements with two companies. This legal dispute highlights the immediate tangible profit that can be gleaned with the help of biotechnology, and the ethical and legal challenges that we face. It also suggests the trust, or better distrust, between the public and the medical research community. Scientists must become accustomed to the fact that the public eye begins to scrutinize ongoing research closely and is less willing to wait for the final results. I was recently startled to see a news reporter monitor one of our otherwise tranquil research seminars that dealt with a potential drug against AIDS. After initial resentment over losing the scientific privacy of the collegium, I began to realize the importance of conveying scientific ideas and therapeutic developments in a fashion that can be understood by the general public. I have since learned that nonscientists are keen on hearing the inside stories on alleged medical breakthroughs, which can elicit confusion and anxiety among the less well informed. The advent of biotechnology and novel therapeutic agents provides the biomedical-pharmaceutical scientist with a unique opportunity to bridge the gap through better communication. While one should avoid premature announcements of cures (remember the public blitz with interferon?), silence will be equally damaging. At least, there does not appear to be any lack of public interest.

Few therapeutic agents have attracted as much attention as the interferons, initially hailed as the new magic bullets against cancer. Their turbulent recent history sets an example for the type of sensationalism that scientists, reporters, and the public should avoid. Early experiments were performed with rather impure interferon preparations until the cloned interferon products became available. During this initial time period, expectations were inflated to the point where the rather meager first clinical results inexorably led to disillusionment. Interferon actually represents a family of related glycoproteins (α, β, γ-interferon) that are secreted by fibroblasts and leukocytes. The fact that degly-

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0724-8741/86/0200-0003$05.00/0

cosylation does not affect interferon's antiviral activity is important in view of the unglycosylated proteins obtained by genetic engineering. Nevertheless, the full significance of post-translational modification within the cell remains poorly understood and reflects one of the many complexities associated with proteins as drugs. It is now clear that interferon may play some role, possibly as an adjunct, in cancer therapy, but that it falls far short of a panacea. Most protein drugs possess multiple effects on tissues, with the interferons providing a good example. Being first discovered as an antiviral substance, α-interferon as a nasal spray was found to reduce significantly the incidence of common colds, according to Dr. F. Hayden and his colleagues from the University of Michigan. His study could mark the first significant progress against these pervasive infections. Further, Dr. L. Jacobs and colleagues from Buffalo reported a marked reduction in disease symptoms of multiple sclerosis patients treated intrathecally with β-interferon over a 4-year period, again a possible advance against this previously untreatable disease. γ-Interferon is being tested against rheumatoid arthritis in a Biogen (Cambridge, Massachusetts) directed study. Hence, many more years may be required to evaluate the full therapeutic potential of the interferons.

Another hot spot among the protein drugs are the interleukins, especially interleukin-2 (IL-2), a T-cell growth factor that stimulates killer cell activity. Together with α-interferon, IL-2 is one of the major products of Cetus Corporation with focus on cancer chemotherapy. Steven A. Rosenberg, Chief of Surgery at the NCI, and his colleagues recently reported good responses and even a complete remission in patients with melanoma, colorectal, kidney, and lung cancer, all difficult to treat. His novel approach includes treating lymphocytes extracted from the patient's blood with IL-2, in the hope of stimulating killer cell activity against cancer cells, and reinjecting the cells together with additional IL-2 into the patients. While some have labeled these results as a "breakthrough against cancer," the procedure is costly and complicated, and significant side effects are observed. In contrast to IL-2, interleukin-1 has only very recently been cloned in two varieties (α and β), but it also holds considerable clinical promise. Il-1 is released from monocytes in response to inflammatory challenge and activates IL-2 producing lymphocytes. It hence resides at an earlier link of the lymphokine chain. Potent growth-promoting properties for multiple hemopoietic cell lineages are attributed to the 140 amino acid peptide IL-3, a T-lymphocyte derived kinine. Its complete chemical synthesis with the solid-phase technique of Merrifield was recently reported by Clark-Lewis and colleagues from Cal Tech. The ability to synthesize peptides of this length opens countless possibilities of modification and definition of the active sites on the molecule, an undertaking that can also be accomplished with recombinant DNA methods (cloning of restriction fragments, site-specific mutations).

The known lymphokines, which include the interleukins, and tissue growth factors are rapidly increasing in number: erythropoietin, granulocyte-macrophage CSF (colony stimulating factor), hepatocyte stimulating factor, epidermal growth factor, nerve growth factor, tumor necrosis factor, and many more. All of these factors play key roles in hemopoiesis, regulation of the immune system, nerve growth, and the development of neural connections, and are likely to affect the course of diseases. I am most fascinated by recent discoveries how these proteins can serve as links between major endocrine, neural, and metabolic systems, thus providing a fresh glimpse of how the body as a whole reacts to stress and disease. For example, the hepatocyte stimulating factor which is released by monocytes was shown to stimulate ACTH release in pituitary cells, thereby providing a new axis between monocytes and adrenal cortical cells. Another profound advance in our understanding of cell proliferation, differentiation, and neoplastic transformation could result from the newly discovered link between the tissue growth factors and some of the cellular oncogene products. The cytoplasmic oncogene encoded products may be essential in processing the information carried by growth factors to the cell and may thus mediate or facilitate the cell's response. In some cases, oncogenes may represent altered protooncogenes that could make the cell independent of the growth factor. For example, the *erb*B oncogene is thought to represent an altered receptor for epidermal growth factor. Understanding of these fundamental processes could become crucial in the development of future cancer therapies.

Tumor necrosis factor (TNF) is currently being tested as an anti-neoplastic agent, with some encouraging results. TNF-α is produced by mitogen-stimulated macrophages, TNF-β by lymphocytes. However, the effect on tumor cells is rather selective, with some cells showing no response and normal fibroblasts even augmented growth. One realizes that factors such as TNF have multiple actions on many cells, and selective cancer chemotherapy may be feasible only in a select few cases. TNF-α has also been combined with γ-interferon, which gave synergistic kill against some cell lines, according to researchers at Genentech. The possibility or even necessity of combining two or more factors results in a quantum leap in the complexity of any therapeutic regimen that may arise from these studies.

Seven hundred fifty thousand patients suffering from heart attack in the USA alone could benefit from tissue plasminogen activator (TPA). Singled out by Genentech as its product of greatest therapeutic and commercial value close to FDA approval, and in large-scale production licensed to Boehringer Ingelheim, Activase (TPA) has shown superior ability to dissolve blood clots *in vivo* without noticeable side effects. Severe competition from other drug houses is assured, and one must now await the potential benefits in large-scale clinical application. Zivin and colleagues in a recent *Science* article noted that TPA also reduces neurological damage after cerebral embolism in experimental animals. Thus, TPA may also become useful in the treatment of embolic stroke. Of similar general importance could be angiogenin, which was painstakingly isolated from colon adenocarcinoma cells by B. L. Vallee and co-workers of Harvard University. It is thought to play a crucial role in the angiogenesis of coronary arteries, would healing, and embryonic development and may be useful in diabetic retinopathy. Possibly of even greater therapeutic importance, the inhibition of angiogenin's function could halt the growth of solid tumors which are dependent on the development of new blood vessels.

Guided by morphological studies on atrial cardiocytes with secretory features, A. J. de Bold of Kingston, Ontario, Canada, characterized a novel hormone-like peptide that

shows potent diuretic, hypotensive, and inhibitory effects on renin and aldosterone secretion. Dubbed atrial natriuretic factor (ANF), these peptides of 2,500 to 13,000 Daltons (storage and release forms) regulate plasma volume and may become useful in combating hypertension. However, Wangler and colleagues recently noted that Atriopeptin-II (a form of ANF) also serves as a potent coronary vasoconstrictor and reduces cardiac output *in vivo,* which again highlights the multifunctionality of most of these protein factors and the need for caution in therapeutic applications.

The production of follicle stimulating hormone (FSH) is inhibited by a reproductive protein, named inhibin by R. Guillemin and co-workers from the Salk Institute of La Jolla. As FSH is needed to produce eggs or sperm, and as inhibin selectively blocks FSH and not LH (which indirectly controls sex drive in man), it could yield a hormone-based male (or female) contraceptive.

I cannot even begin to cover the therapeutic and diagnostic potential of genetically engineered immunotoxins, antigens as vaccines, and monoclonal antibodies (MAB). With cytotoxic effects of their own against certain T-lymphomas as an example, MABs can also serve to carry toxins or drugs to target cells with specific surface markers. Diener and colleagues from Edmonton, Alberta, Canada, have attached the cytotoxic drug daunorubicin via an acid-sensitive spacer that is cleaved in the acid environment of the lysosomes after internalization of the MAB complex into the target cell. Several similar approaches have been successful in targeting cytotoxic agents toward tumor cells *in vitro,* but their therapeutic value is usually less clear-cut *in vivo.* Hybridoma technology combined with recombinant DNA techniques can be used to produce chimeric MABs with a human constant region (Fc) fragment and a mouse variable (Fab) fragment in order to suppress the immune response to MAB in patients, which represents one of the problems encountered in cancer trials.

Protected against the immune response of the host by constant modulation of their surface antigens, many parasites appear resistant to our attempts to develop effective vaccines. However, it was recently possible to pinpoint invariable epitopes on surface proteins of *Plasmodium vivax.* Arnd and colleagues described a nonapeptide fragment on a surface protein that is present in multiple tandem repeats and constant among *P. vivax* strains. The synthetic 18 amino acid peptide binds to the surface protein's antibody which has been shown to provide protective immunity. Hence, such synthetic peptides may represent the target epitopes of acquired immune protection and could serve as vaccines. This nonapeptide, however, is not shared by *P. falciparum,* which may express a different set of multiple repeat peptide epitopes. A similar problem arises with the development of vaccines against the virus implicated in acquired immune deficiency syndrome (AIDS). Because of the genetic variability of the HTLV/LAV virus, multivalent antibodies may be required for protection. Having mapped the entire genome of this virus, scientists can now search for immunogenic proteins that are expressed by all variants. Among the different classes of HTLV-III encoded antigens, the *gag*- and *env*-encoded products appear to be most promising, since antibodies against these antigens are consistently found in AIDS patients. These immunogenic proteins are thus targets for diagnostic tests and effective vaccines.

After this much publicity over potential cures, one feels disappointed with the number of genetically engineered substances that have actually reached the market (Table I). At present the list includes only two well known peptide hormones, human insulin and human growth hormone. Both have been cloned early on by researchers at Genentech as obvious targets of the newly emerging recombinant DNA technology, while the peptide somatostatin was the first human hormone to be expressed in a microorganism. The Genentech-developed insulin is marketed by Eli Lilly as Humulin, one of the many licensing examples between large drug houses and biotech companies. With growth hormone, Genentech has initiated its own production and marketing under the name of Protopin. It has just received Orphan Drug Status from the FDA, which applies to diseases affecting no more than 200,000 people in the USA and grants Genentech a seven-year monopoly on the drug's sales. At least 4000 children in the USA need growth hormone to achieve normal growth, and possibly many more with less severe growth deficiencies might also benefit from the hormone. The insulin market is of course much larger, resulting in stiff competition among genetically engineered insulins from a number of companies. Similarly, the interferons, interleukins, and tissue plasminogen activator are each being produced by several companies, which shifts the emphasis of the competition from the actual production process to marketing.

Without any doubt, the novel protein drugs could have a profound effect on therapy in the foreseeable future. However, if there is indeed a revolution in therapy, we are clearly only at the beginning. While new results arrive at a rapid pace, nature is just a little more complex than initially thought. These new cell signal proteins are unlikely to yield miracle drugs soon. And even if they do, let us not forget that the magic bullets of some 40–50 years ago, the antibiotics and sulfonamides, have done little to increase the overall longevity of mankind, which is apparently propelled to unequal lifespans by factors other than drugs. Nevertheless, for the individual patient whose health or life depends on effective therapy, these agents could bring the miracle of normal life—for example, to children treated with Protopin

Table I. Current Status (End of 1985) of Protein Drugs Produced by Genetic Engineering Techniques and Hybridoma Technology

Marketed	Human insulin
	Human growth hormone
FDA approval filed	α-Interferon
In clinical trial	
Phase III	Hepatitis B vaccine
Phase II	β-Interferon
	γ-Interferon
	Immunotoxin-melanoma
	Interleukin-II
	Tissue plasminogen activator
	Retrovirus vaccine
Phase I	Various anticancer antibodies (pancreatic, colorectal, lymphomas)
	Epidermal growth factor
	Malaria vaccine
	Tumor necrosis factor

and to heart attack victims if treated successfully with Activase.

What do the protein drugs mean to the pharmaceutical scientist who stands at the crossroads between the physicochemical and biological sciences? The answer seems obvious. Therapy with these proteins will be optimized only if one understands the mechanism of action, metabolism, physicochemical characteristics, the effects of altered protein structure on their biological functions, the heterogeneity of individual factors, their stability, problems of their production, and last but not least their proper packaging into a therapeutic delivery system. In other words: virtually unlimited hunting grounds for researchers in the biomedical-pharmaceutical field. As educators in the pharmaceutical disciplines, we have to tackle the question of how and to what extent should we bring this new area to our graduate and professional students. Opportunities abound, and molecular biologists have taken full advantage to move their field ahead. Pharmaceutical scientists need to react quickly in order to seize the research opportunities in this newly emerging area.